993-Symp
Computational Insights Into Cytoskeletal Rheology
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Cytoskeletal rheology is critical in the interaction of cells with their environment. It influences cell migration, mechanotransduction, and the ability of the cell to transmit externally applied forces. Models for cell mechanics have progressed considerably in recent years, and are now capable of simulating the effects of thermal fluctuations, cross-linking, and stresses generated by motor activity, all thought to be important determinants of cytoskeletal viscoelastic behavior. At the same time, model systems such as reconstituted actin gels have been used to gain insight into the factors that give rise to the unique cytoskeletal behavior. Of interest in this regard are: (i) the unique power-law rheology observed in the linear stress regime, (ii) nonlinear strain stiffening, (iii) fluidization that occurs above some critical level of stress, beyond which the modulus falls precipitously, and (iv) the role of prestress on cell stiffness. Here we present results from recent models that address each of these phenomena. We show through modeling that (i) thermal fluctuations of actin filaments are important only at low levels of prestress and small values of the ratio of persistence length to distance between cross-links, (ii) power-law rheology appears to emerge as a consequence of motor-induced prestress and cross-links, (iii) in the strain-stiffening regime, prestress is the primary determinant of network modulus, (iv) cross-link rupture rather than unfolding accounts for network remodeling and stress relaxation behavior, (v) motor activity generates prestress, but also has a strong influence on network morphology and the formation of stress fibers. [Support from the Sumsung Scholarship Program (to TYK) and the NIH (GM076689) is gratefully acknowledged.]

MINISYMPOSIUM 2: Store-Operated Calcium Channels: New Directions

996-MiniSymp
CRAC Channel Function Through ORAI1, STIM1 and STIM2 is Required for Proinflammatory Effector T Cell Function in Autoimmunity
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ORAI1 constitutes the pore forming subunit of the Ca2+ release activated Ca2+ (CRAC) channel, which is responsible for store-operated Ca2+ entry (SOCE) in many cell types. ORAI1 is activated by stromal interaction molecules (STIM) 1 and 2 following immunoreceptor triggering and depletion of ER Ca2+ stores. ORAI1, STIM1 and also STIM2 are essential mediators of SOCE and CRAC channel function in human and murine T lymphocytes. A role for ORAI1 and STIM1 in T cells in vivo has been inferred from in vitro studies of cells from human immunodeficient patients with mutations in ORAI1 and STIM1 and mice with targeted deletion of Orai1, Stim1 and Stim2 genes. Investigating the role of SOCE in T cells in the context of autoimmunity and inflammation, we find that mice with T-cell specific deletion of Stim1 or Stim2 are protected from experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. T cells lacking STIM1 or STIM2 failed to produce the proinflammatory cytokines IL-17 and IFNγ, which are crucial for the induction of EAE. SOCE is also required for the proliferation and homeostasis of IL-17 producing Th17 cells. ORAI1 is similarly critical for several aspects of T cell mediated immunity in vivo. Immune responses that depend on Th1, Th2 and Th17 cells were severely attenuated in Orai1-deficient mice expressing a non-functional form of the CRAC channel. Orai1-deficient mice tolerated skin allotransplants significantly longer than wildtype littermates. Furthermore, T cells from Orai1-deficient mice failed to induce colitis in a T-cell dependent adoptive transfer model of inflammatory bowel disease as they lacked production of IL-17, IFNγ and TNFα. Taken together, our findings demonstrate that ORAI1, STIM1 and STIM2 are critical mediators of proinflammatory T cell function and autoimmunity.

997-MiniSymp
Stoichiometric Requirements for Trapping and Activation of CRAC Channels by STIM1 At ER-Plasma Membrane Junctions
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Store-operated Ca2+ entry is regulated by physical interactions of the ER Ca2+ sensor STIM1 and the CRAC channel protein Orai1. Recent studies support a diffusion-trap mechanism in which Ca2+ depletion from the ER causes STIM1 to accumulate at ER-plasma membrane (ER-PM) junctions, where its CRAC activation domain (CAD) binds to Orai1, trapping and activating mobile CRAC channels in the overlying PM. The ability of the CAD to cluster CRAC channels suggests that each channel contains multiple STIM1 binding sites, but the number of sites that must be occupied to trap and activate CRAC channels is unknown. By competing for a limited amount of STIM1, increasing levels of Orai1 are expected to reduce the number of STIM1s bound per channel until the minimum stoichiometry sufficient for channel trapping is reached. In HEK293 cells expressing a fixed amount of mCherry-STIM1 and increasing levels of GFP-Orai1, the junctional STIM1:-Orai1 ratio reached a minimum of ~0.3; thus, binding of 1-2 STIM1 appears sufficient to immobilize tetrameric CRAC channels at ER-PM junctions. In cells expressing a constant amount of STIM1, CRAC current (ICRAC) was a highly nonlinear bell-shaped function of Orai1 expression, and the minimum STIM1:Orai1 stoichiometry for channel trapping failed to evoke significant activation. A simple cooperative gating model fitted to the data suggests that while STIM1 binding to a single site is sufficient to trap CRAC channels, four sites must be occupied to cause significant activation. This highly nonlinear behavior supports conclusions based on current fluctuation analysis (Prakriya and Lewis, J. Gen. Physiol. 128:373-86, 2006) that the slow development of whole-cell ICRAC after store depletion reflects the stepwise recruitment of individual channels from a silent to a high open-probability state as they enter ER-PM junctional sites.

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Definition of STIM1 Phosphorylation Sites that Contribute to Suppression of Store-Operated Calcium Entry During Mitosis
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STIM1 is an essential protein for store-operated calcium entry (SOCE), whereby Ca^{2+} influx into the cell is activated in response to depletion of intracellular Ca^{2+} stores in the endoplasmic reticulum (ER). In response to store depletion, STIM1 rearranges to sites at which the ER membrane is closely juxtaposed with the plasma membrane, where STIM1 interacts with and gates the Orai Ca^{2+} influx channels. We recently demonstrated that during mitosis, STIM1 is phosphorylated at multiple serine and/or threonine residues recognized by the phosphospecific MPM-2 antibody, and that this phosphorylation is responsible for mitosis-specific suppression of STIM1 rearrangement and SOCE. However, the physiological relevance of STIM1 phosphorylation and SOCE suppression for the process of cell division remains unclear. To this end, we have begun to define all the mitosis-specific phosphorylation sites of STIM1. We have simultaneously mutated to alanine 9 out of the 10 putative MPM-2 sites (one results in loss of expression), which resulted in complete loss of MPM-2 recognition of mitotic STIM1. This 9-site mutant also afforded a significant rescue of STIM1 rearrangement and SOCE in mitotic cells, suggesting that phosphorylation of at least a fraction of these sites contributes to SOCE suppression. We then individually reversed each of the 9 alanines back to serine, which revealed that S486, S600, S628, and S668 are phosphorylated during mitosis and contribute to MPM-2 recognition. However, simultaneously mutating these 4 serines to alanine did not completely abolish MPM-2 recognition, nor did it rescue as well as the 9-site mutant, suggesting that additional sites remain to be identified. Once we have identified these additional sites, we will have the tools necessary to begin deciphering the importance of STIM1 phosphorylation for the integrity of cell division and cell cycle regulation.

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Cooperativeness of Orai Cytosolic Domains Tunes Subtype-Specific Gating
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Activation of immune cells is triggered by the Ca^{2+} release-activated Ca^{2+} (CRAC) current, which is mediated via channels of the Orai protein family. A key gating process of the three Orai channel isoforms to prevent Ca^{2+} overload is fast inactivation, most pronounced in Orai3. A subsequent reactivation is a unique gating characteristic of Orai1 channels, while Orai2 and Orai3 currents display a second, slow inactivation phase. Employing a chimeric approach by sequential swapping of respective intra- and extra-cellular regions between Orai1 and Orai3, we show here that Orai1 specific proline-arginine-rich domains in the N-terminus mediate reactivation, while the second, intracellular loop modulates fast and slow gating processes. Swapping C-terminal strands lacks a significant impact. However, simultaneous transfer of Orai3 N-terminus and its second loop or C-terminus in an Orai1 chimera substantially increases fast inactivation centered between wild-type channels. Concomitant swap of all three cytosolic strands from Orai3 onto Orai1 fully conveys